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The first DNA-supported record of *Platicrista angustata* (Eutardigrada, Hypsibiidae) from Germany and reply to Scheirer et al. (2024)

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Abstract

The recent paper of Gąsiorek et al. (2024a), containing integrative redescriptions of several taxonomically important hypsibiid species, provided necessary discriminative criteria used in modern eutardigrade identification. Herein, I present first DNA-backed, reliable record of a rare species *Platicrista angustata* from the German Taunus mountains. I also indicate that statements of Scheirer et al. (2024) are completely unfounded and result from the general lack of understanding what Gąsiorek et al. (2024a) wrote. Moreover, Scheirer et al. (2024) introduced new mistakes and misconceptions regarding *Platicrista*. Consequently, I provide a concise and full diagnosis of the genus *Platicrista*.

Key Words

distribution data, DNA barcoding, Hypsibioidea, peer review responsibility

Introduction

The genus *Platicrista* was established by Pilato (1987) based on the shape of the apophyses for the insertion of the stylet muscles (AISMs) and the shape of stylet furcae. Most *Platicrista* spp. are among the largest eutardigrades, easily reaching over 800 µm in body length (Gąsiorek et al. 2024a), and the genus comprises 11 valid spp. (Degma and Guidetti 2024) after the abolition of *Meplitumen* (Tumanov and Tsvetkova 2023). This short note delivers the first sound report of the type species *P. angustata* (Murray, 1905) from Germany and shows that criticism of the paper by Gąsiorek et al. (2024a) done by Scheirer et al. (2024) is substantially flawed and misdirected. The errors of Scheirer et al. (2024) are scrutinised and rectified; a short, concise and meaningful genus diagnosis is proposed as a result.

Materials and methods

Several moss and lichen samples were collected by Katarzyna Vončina in the Taunus mountain range on 9th April 2023; one sample (50°13'09"N, 8°29'55"E, 622 m asl; Weiße Mauer, moss from tree trunk) contained three specimens representing the genus *Platicrista*. Tardigrades were extracted from the sample according to the procedure from Degma (2018) and provisionally identified as *P. angustata* based on the morphological comparison of one permanently mounted (Hoyer's medium was applied) individual with its redescription (Gąsiorek et al. 2024a). The slide DE.291.01 was examined under an Olympus BX53 phase contrast microscope associated with an Olympus DP74 digital camera. The remaining two individuals were processed for molecular analysis to obtain a confirmation of species' identity. DNA was extracted from single tardigrades using Chelex® 100 resin (Casquet et al. 2012). A single hologenophore was mounted in Hoyer's medium after extractions (Pleijel et al. 2008). Four DNA markers were sequenced (18S rRNA, 28S rRNA, ITS-2, COI) according to the protocols from Suppl. material 1. Sequences were processed using BioEdit v.7.2.5 (Hall 1999). MEGA v.7 (Kumar et al. 2016) was used to calculate uncorrected *p*-distances.

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The most updated, four-marker based hypsibiid dataset (Suppl. material 2; Gąsiorek et al. 2024b) and new sequences for *Platicrista* were aligned using default settings of MAFFT v.7 (Katoh et al. 2002; Katoh and Toh 2008), with *Calohypsibius ornatus* (Richters, 1900) used as an outgroup. All gaps were left intact after manual checking in BioEdit. COI alignment was divided into three data blocks constituting three separate codon positions before partitioning. The sequences of all gene fragments were concatenated to generate a matrix of 2768 bp in SequenceMatrix (Vaidya et al. 2011). Based on PartitionFinder v.2.1.1 (Lanfear et al. 2017), under the Bayesian information criterion (BIC) and greedy algorithm (Lanfear et al. 2012), the best scheme of partitioning and substitution models were chosen for posterior phylogenetic analysis. Six predefined partitions were inferred as suitable for the dataset, and the best-fitting model was GTR+I+G for all. Bayesian inference (BI) marginal posterior probabilities were calculated with MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). Random starting trees were used, and the analysis was run for 10 million generations, sampling the Markov chain every 1000 generations. An average standard deviation of split frequencies of < 0.01 was used as a guide to ensure that the two independent analyses had converged. The program Tracer v.1.3 (Rambaut et al. 2014) was then used to ensure that Markov chains had reached stationarity and to determine the correct burn-in for the analysis, which was the first 10% of generations. The effective sample size values were $\gg 200$. A consensus tree was obtained after summarizing the resulting topologies and discarding the burn-in. ModelFinder (Kalyaanamoorthy et al. 2017) was used to choose the best-fitting models for six partitions in maximum likelihood (ML): TIM3e+I+G4 (18S rRNA), GTR+F+I+G4 (28S rRNA), TPM2u+F+I+G4 (ITS-2), SYM+I+G4 (CO1), GTR+F+G4 (CO2), and HKY+F+I+G4 (CO3), chosen according to the BIC. W-IQ-TREE was used for ML reconstruction (Nguyen et al. 2015; Trifinopoulos et al. 2016). Ten thousand ultrafast bootstrap (UFBoot) replicates were applied to provide support values for nodes (Hoang et al. 2018). All final BI and ML consensus trees were visualized by FigTree v.1.4.3 (available from http://tree.bio.ed.ac.uk/software/figtree).

Hypsibiid systematics follows Bertolani et al. (2014) and Gąsiorek et al. (2024a). Terminology for leg structures follows Gąsiorek et al. (2024a).

Results

The overall hypsibiid tree topology reflected that from the recently published phylogenies (Tumanov and Tsvetkova 2023; Gąsiorek et al. 2024a, b). *Platicrista* was again found in insufficiently supported clade with *Astatumen* (Fig. 1), and the two German specimens formed a clade with formerly sequenced representatives of *P. angustata* from Scotland and Norway (Fig. 1). This corresponds well with no differences between them in two markers (18S rRNA: PQ799486–7; ITS-2: PQ805168–9) and minor *p*-distances in the other two (28S rRNA: 0.1%, PQ799494–5; COI: 1.5–2.0%, PQ799496–7). All three German individuals were longer than 500 µm and fully conformed with the morphotype of *P. angustata* depicted in Gąsiorek et al. (2024a).

Discussion

This note positively verifies the presence of *P. angustata* in Germany after its neotype was established from Scotland and a complex of several similarly looking species disclosed (Gąsiorek and Michalczyk 2020; Gąsiorek et al. 2024a). Recently, Scheirer et al. (2024) published a critique of my paper, in which they confused redescription of *P. angustata* done in Gąsiorek et al. (2024a) with amended definition/diagnosis of the entire genus (not performed in the same work). Consequently, all allegations of presumed errors in Gąsiorek et al. (2024a) formulated by Scheirer et al. (2024) are pointless since they refer to something that was not included in my work. Moreover, Scheirer et al. (2024) incorrectly stated that *P. itaquasconoide* (Durante Pasa & Maucci, 1975) was synonymised with *P. angustata*, when in reality it was assigned a *nomen inquirendum* status.

It should be stressed that Gąsiorek et al. (2024a) corrected the description of *P. brunsoni* Miller and J.D. Miller, 2021 based on the microphotographic documentation from the original work and a fresh material from Wyoming. Unfortunately for the critics, they advanced another series of errors in the description of *P. loloensis* Scheirer et al., 2024, which is a valid species, and other remarks formulated on the genus. The mistakes were as follows:

1. *Platicrista loloensis* does not exhibit median cuticular bars since these were defined for legs I–III. An adequate term for structures present in this species is "posterior bars", as drawn in fig. 1 for claws IV in Gąsiorek et al. (2024a).
2. *Platicrista* certainly does not show "a largely Holarctic distribution", as stated by Scheirer et al. (2024). *Platicrista borneensis* Gąsiorek & Michalczyk, 2024 and *P. nivea* Gąsiorek, 2024 (both described in Gąsiorek et al. 2024a) occur in the Oriental region, and there are Neotropical *Platicrista* records as well (Maucci 1988; Marley 2006; Ramsay et al. 2021; Gąsiorek et al. 2024a). Therefore, the newest data contradict the previously assumed Holarctic geographic range of *Platicrista*, as "Holarctic" does not mean "north of equator", which seems to be the conviction of Scheirer et al. (2024).
3. Finally, the amended genus diagnosis proposed by Scheirer et al. (2024) is overly long and embraces numerous characters typical for many hypsibiids and/or other eutardigrades (e.g. blunt head, the position of mouth opening, short and plump legs, the absence of eyes), so it does not fulfil the criterion

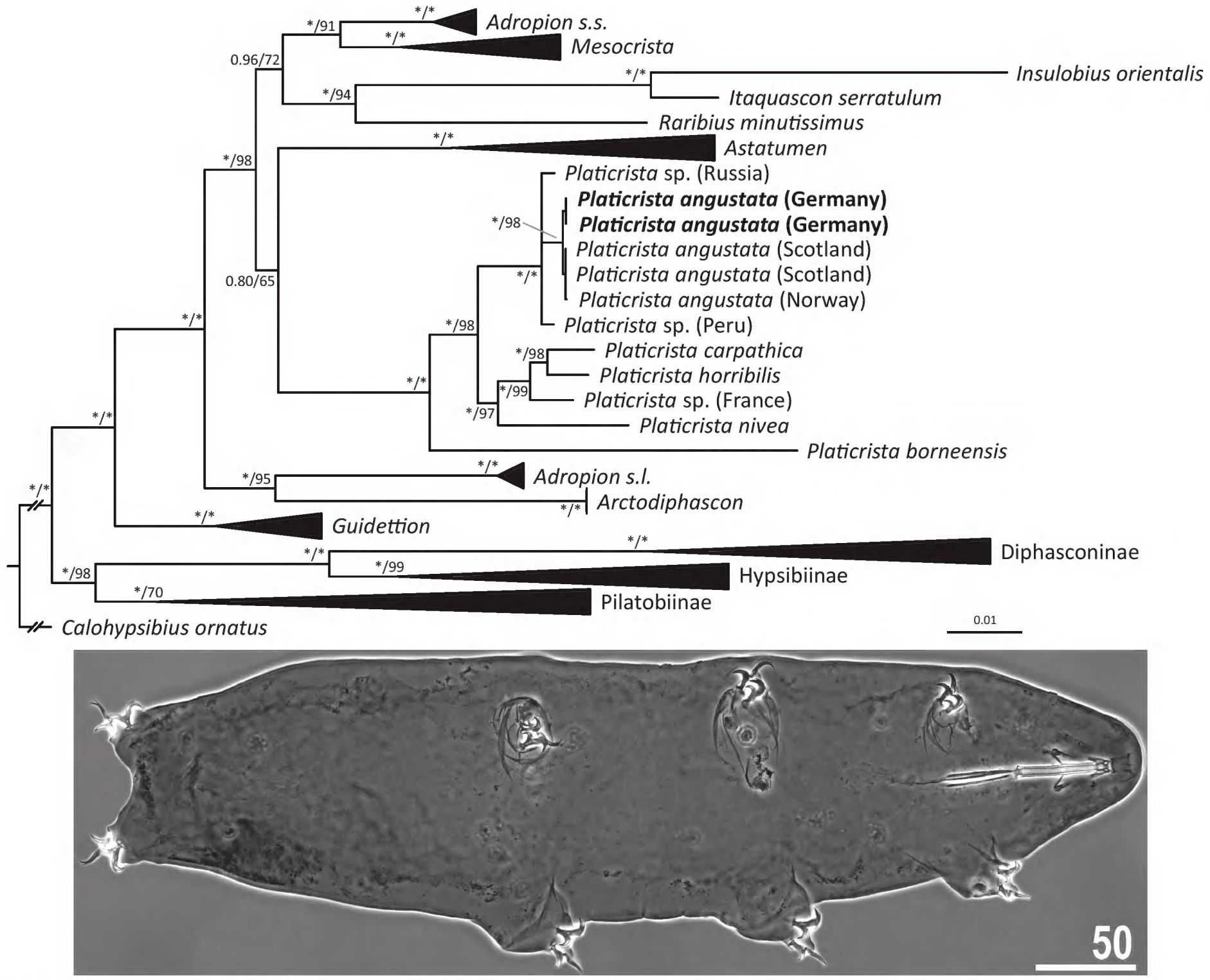

Figure 1. Phylogenetic relationships within Hypsibiidae in the Bayesian and Maximum Likelihood analyses (detailed congruent topologies identical as in Gąsiorek et al. 2024a, b). The scale refers to the Bayesian tree and represents substitutions per position; posterior probability values are provided before slash above the nodes, whereas bootstrap values can be found after slash; *, maximal support. *Calohypsibius ornatus* constitutes an outgroup. New record (*P. angustata* illustrated below; scale bar in μm) in bold.

of diagnosability (Borkent 2021). Moreover, the addition of descriptive characters to claws (large and robust, etc.) is wrong since those epithets exclude *P. borneensis* having delicate and non-massive claws (Gąsiorek et al. 2024a). Therefore, I present a brief and unequivocal, amended diagnosis of *Platicrista* based on the previous findings and the current understanding of character relevance in the Hypsibiidae (Gąsiorek and Michalczyk 2020; Tumanov and Tsvetkova 2023; Gąsiorek et al. 2024a, b): "Cuticle smooth or irregularly wrinkled/granulated in the posterior body portion; rarely covered with flat polygonal tubercles. The AISMs wide and flat, symmetrical with respect to the frontal plane. Buccal tube may be annulated but typically smooth; it is followed by a flexible annulated pharyngeal tube; the pharyngeal annulation is double (the annuli form pairs – dyads). A dorsoposterior apodeme of the buccal tube (DABT) is absent. An ovoid pharynx with two macroplacoids in the form of thin cuticular ridges arranged in parallel; microplacoid/septulum absent. Stylet supports present and straight, inserted high on the buccal tube. Stylet furcae strongly modified, spoon-like laterally. Claws of the *Hypsibius* type, i.e. asymmetrical both with respect to the sequence of primary and secondary branches (2-1-2-1) and with respect to their size, with external and posterior claws always evidently larger than the internal and anterior claws. Pseudolunulae below claws I–IV may be present; cuticular bars of various types (internal, median, posterior) present or legs without bars.".

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Supplementary material 1

Primers and references for PCR programmes

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Supplementary material 2

GenBank accession numbers

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